Assessment of the risk factors determining the prognosis of major and minor limb amputations in patients with diabetic foot ulcers

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Abstract

Background. Diabetes mellitus (DM) is a major global health problem, and its incidence is growing. Depending on this increase, the number of diabetes-related complications will also rise.

Objectives. This study aimed to determine the risk factors associated with major and minor amputations resulting from diabetes.

Materials and methods. Patients diagnosed with diabetic foot complications (n = 371) and hospitalized between January 2019 and March 2020 were retrospectively evaluated using information obtained from the database of Diabetic Foot Wound Clinic. Examination of the data identified 165 patients for inclusion in the study, who were stratified into major amputation (group 1, n = 32), minor amputation (group 2, n = 66) and non-amputation (group 3, n = 67) groups.

Results. Of the 32 patients who underwent major amputations, 84% had a below-knee amputation, 13% had an above-knee amputation and 3% had knee disarticulation. At the same time, 73% of 66 patients who underwent minor amputation had a single-finger amputation, 17% had a multiple-finger amputation, 8% had a transmetatarsal amputation, and 2% had Lisfranc amputation. Laboratory results showed high acute phase protein and low albumin (ALB) levels in patients from group 1 ($p < 0.05$). Although *Staphylococcus aureus* was found to be the most common infectious agent, Gram-negative pathogens were dominant ($p < 0.05$). Also, there was a significant cost difference between the groups ($p < 0.05$). Furthermore, those aged over 65 had a high Wagner score, high Charlson Comorbidity Index (CCI), long diabetic foot ulcer (DFU) duration, and high white blood cell (WBC) count, all of which were risk factors for major amputation ($p < 0.05$).

Conclusions. This study demonstrated an increased Wagner staging and incidence of peripheral neuropathy (PN) and peripheral arterial disease (PAD) in major amputation patients. In addition, the rate of distal vessel involvement was high in major amputation patients, with elevated acute phase proteins and low ALB levels crucial in laboratory findings.

Key words: prognosis, risk factors, cost, amputation, diabetic foot ulcers

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Background

Diabetes mellitus (DM) is a significant global health concern that is increasing in incidence.[1–3] The number of diabetes-related complications is also rising,[2,3] which carries an economic burden.[1–4]

One of the most disabling complications of DM is diabetic foot ulcers (DFUs), which result from various etiological pathways.[1–6] It is estimated that 15–25% of diabetics will be affected by DFUs at some point in their lives,[1,4–7] and their recurrence is also common, with 70% of patients experiencing recurrent lesions within 5 years of treatment.[1] Moreover, the risk of death after 5 years is 2.5 times higher for a patient with a DFU than for diabetes patients without a DFU.[1] However, the most undesirable potential outcome of DFU, other than death, is lower extremity amputation (LEA).[1,3,7,8]

Amputations due to DFUs are the most common cause of non-traumatic amputations.[7–9] After the 1st amputation, the incidence of a 2nd in the contralateral limb approaches 50% within 2 years.[4,10] In addition to these risks, the medical and psychosocial consequences of LEAs are substantial.[1,7] In this regard, DFU and LEA patients have a significantly reduced quality of life and a higher risk of depression, which may be associated with impaired psychosocial functioning.[1,7]

Diabetic foot ulcers are difficult to treat and often long-term, taking weeks or months to heal, and they may not heal at all.[1] Early diagnosis and treatment of DFUs is vital because of the increasing prevalence of diabetic patients and the growing health burden.[2] Therefore, identifying the risk factors for the prognosis of patients with DFU and those at high risk, as well as taking preventative action, can reduce complications that may develop.[11]

Many risk factors have been identified for DFU development.[1–5,8,9,12,13] Those identified in previous studies include diabetic peripheral neuropathy (PN), infection, peripheral arterial disease (PAD), chronic renal failure (CRF), advanced age, male sex, smoking, foot deformities, poor glycemic control, large ulcer size, hypertension, lipid abnormalities, and comorbidities, along with elevated white blood cell (WBC) count, plasma albumin (ALB), glycosylated hemoglobin A1c (HbA1c), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).[1–5,8,9,12–17] However, results from studies on these DFU risk factors are inconsistent.[1–4,11,12]

Lower extremity amputations due to DFU are generally defined as major or minor amputations,[3,4,8,9] and there is a strong association between the type of amputation and the future functional capacity of patients.[4,8,9] However, there are few studies comparing risk factors between major amputation, minor amputation and non-amputation patient groups.[4,8,9,13]

Objectives

This study aimed to determine the clinical differences and risk factors between major amputation, minor amputation and non-amputation patient groups to reduce the possible amputation risk, increase treatment efficiency in DFU patients and develop better treatment strategies.

Methods and materials

Patients

The study retrospectively evaluated 371 patients hospitalized with a DFU diagnosis between January 2019 and March 2020. The data were obtained after examining the database of the Kayseri City Hospital Diabetic Foot Wound Clinic (Kayseri, Turkey). From initially assessed patients, 165 (110 males and 55 females; 94 right-sided and 71 left-sided; mean age: 64.87 ±11.82 years; range: 42–92 years) were included in the study.

Exclusion criteria

Patients who had undergone lower extremity (LE) surgery for any reason were considered for the study. However, those with reduced life expectancy or missing data and patients without a DFU diagnosis were excluded. Also, patients who underwent bilateral amputation, repetitive surgery, or chronic treatment with immunosuppressants or steroids were excluded.

Study design

The patients were divided into major amputation (group 1, n = 32), minor amputation (group 2, n = 66) and non-amputation (group 3, n = 67) groups. A minor LEA was defined as any amputation distal to the ankle joint, whereas a major LEA was understood as any amputation through or proximal to the ankle joint.[18]

Data sources/measurement

Analyzed data included patient age, gender, smoking history, DM duration, DFU duration and side, Wagner classification, amputation history, presence of PN and PAD, laboratory results, microbiologic culture results, length of hospitalization, medical comorbidities, and cost of diabetes care.

Patient comorbidity was evaluated using the Charlson Comorbidity Index (CCI) and the modified CCI (MCCI),[19,20] while the Semmes–Weinstein 5.07 monofilament test assessed PN. Diabetic foot ulcers were classified according to the Wagner system: grade 0 – skin lesions absent, hyperkeratosis below or above bony prominences; grade 1 – skin and immediate subcutaneous tissue are

ulcerated; grade 2 – lesions are deeper and may penetrate to tendons, bone or joint capsule; grade 3 – deep tissues are always involved, osteomyelitis may be present; grade 4 – gangrene of some portion of the toes or forefoot; grade 5 – the entire foot is gangrenous.[21]

Laboratory evaluations included WBC count and hemoglobin (Hb), ALB, plasma creatinine, blood urea nitrogen (BUN), HbA1c, ESR, CRP, and procalcitonin (PCT) levels. The presence of neuropathic arthropathy (Charcot joints) and osteomyelitis were assessed using LE radiographs and magnetic resonance imaging (MRI). The LE Doppler ultrasonography (USG) was used to evaluate PAD. Meanwhile, the dorsalis pedis, tibialis anterior, tibialis posterior, popliteal, and femoral arteries were evaluated for triphasic, biphasic, monophasic, or absence of arterial flow.

Ethical approval

The Kayseri City Hospital Clinical Research Ethics Committee approved the study protocol (approval No. 01.10.2020/166), and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analyses

All data analyses employed IBM Statistical Package for Social Sciences (SPSS) v. 22.0 (IBM Corp., Armonk, USA) software. Percentages and standard deviations (SDs) were determined for categorical data and continuous variables, and the Shapiro–Wilk test, skewness, kurtosis, and histograms were used to evaluate the data distribution. Pearson's χ^2 test compared categorical data between the groups, and analysis of variance (ANOVA) with post hoc tests assessed between-group differences in normally distributed continuous variables. A value of $p < 0.017$ was considered significant in the post hoc analysis. The Kruskal–Wallis test evaluated the relationship between non-normally distributed continuous variables. Multiple linear regression was used for cost analysis after categorizing the factors affecting the cost. The factors affecting the 3 groups were categorized and evaluated with multinomial logistic regression analysis. A value of $p < 0.05$ was considered significant in the multiple linear regression analysis and multinomial logistic regression analysis.

Results

Of the 32 patients who underwent major amputations, 84% (n = 27) underwent below-knee amputation and 13% (n = 4) above-knee amputation, and 3% (n = 1) had knee disarticulation. Meanwhile, 66 patients underwent minor amputation, with 73% (n = 48) undergoing single-finger amputation, 17% (n = 12) multiple-finger amputation, 8% (n = 5) transmetatarsal amputation, and 2% (n = 1) Lisfranc amputation.

Age, ulcer duration, Wagner classification, PN, PAD, CCI, MCCI, and diabetes care cost varied significantly across between the 3 groups ($p < 0.05$). Table 1 summarizes the baseline characteristics of the patients.

Evaluation of laboratory values indicated significantly higher WBC count and CRP and PCT levels, and lower ALB level in group 1 compared to groups 2 and 3. In addition, group 1 had significantly higher ESR and BUN values than group 3 (Table 2). However, there were no significant between-group differences in HbA1c or creatinine values.

Peripheral neuropathy was detected in 69% (n = 114) of patients, with 24 (75%) patients in group 1, 51 (77%) patients in group 2 and 39 (58%) patients in group 3. There was a significant difference between group 1 and group 2 ($p = 0.043$). Doppler USG examination indicated the involvement of at least 1 peripheral artery in 27 (84%) patients in group 1, 33 (50%) patients in group 2 and 26 (38%) patients in group 3 ($p = 0.000$). The involved arteries and the observed flow form are summarized in Fig. 1.

Wound cultures were obtained from 128 patients, with growth detected in 82% (n = 106) of samples. Ten (10%) cultures had polymicrobial growth, and 96 (90%) contained a single microorganism. Microbial growth was detected in 18 of 21 wound cultures in group 1, 47 of 51 in group 2 and 41 of 56 in group 3 cultures. Furthermore, 19 microorganisms were detected in group 1, 52 in group 2 and 45 in group 3.

In the cultures of group 1 patients, 26.3% (n = 5) Gram-positive bacteria and 73.6% (n = 14) Gram-negative bacteria were detected. The most common Gram-positive bacteria isolated were *Staphylococcus* spp. (n = 4), and *Escherichia coli* (n = 4) was the most common Gram-negative bacteria. In group 2, Gram-positive bacterial growth was detected in 34.6% (n = 18) and Gram-negative bacteria growth in 61.5% (n = 32). *Staphylococcus* spp. (n = 9) were the most common Gram-positive bacteria isolated, while *Acinetobacter baumannii* (n = 6) was the most common Gram-negative bacteria. In group 3, Gram-positive bacterial growth was detected in 46.6% (n = 21) and Gram-negative growth in 51.1% (n = 23). The most common Gram-positive bacteria were *Staphylococcus* spp. (n = 12), while *E. coli* (n = 5) was the most common Gram-negative bacteria (Table 3).

Multinomial logistic regression analysis was performed to investigate independent risk factors. The results showed that major amputation was associated with age, WBC count, Wagner classification, DFU duration, and CCI. For minor amputations, male gender, age, Wagner classification, and ESR were crucial risk factors (Table 4).

The mean treatment cost for major amputations was $1023, for minor amputations – $535 and $762 for non-amputations. There was a significant difference between the major amputation and minor amputation groups in terms of mean treatment cost ($p = 0.032$). Age, gender,

Table 1. Demographic characteristics of the patients and clinical outcomes

Patient characteristics		Group 1 (n = 32)	Group 2 (n = 66)	Group 3 (n = 67)	p-value
Age [years], mean (range) ±SD		69.8 (49–92) ±12.1	65.7 (42–86) ±9.4	61.6 (34–87) ±12.8	0.027*[b]
Gender, n (%)	female	10 (31.2)	23 (34.8)	22 (32.8)	0.933[†]
	male	22 (68.7)	43 (65.1)	45 (67.1)	
Side of involvement, n (%)	right	19 (59.3)	41 (62.1)	34 (50.7)	0.397[†]
	left	13 (40.6)	25 (37.8)	33 (49.2)	
Duration of DFU [days], median (range)		30 (6–360)	20 (2–340)	15 (3–360)	0.020[+b]
Duration of DM [years], mean ±SD		18.22 ±9.35	14.97 ±7.87	14.90 ±7.47	0.118*
DM treatment, n (%)	insulin	28 (87.5)	56 (84.84)	54 (80.59)	0.829[†]
	oral antidiabetic drug	4 (12.5)	7 (10.6)	10 (14.92)	
	new diagnosis	0 (0)	3 (4.54)	3 (4.47)	
Mean length of hospitalization [days], median (range)		13 (3–145)	14 (1–69)	12 (1–150)	0.612[+]
Wagner classification, n (%)	grade 1	0 (0)	1 (1.51)	21 (31.34)	0.000[†ab]
	grade 2	0 (0)	2 (3.03)	39 (58.2)	
	grade 3	5 (15.62)	37 (56.06)	7 (10.44)	
	grade 4	8 (25)	26 (39.39)	0 (0)	
	grade 5	19 (59.37)	0 (0)	0 (0)	
Number of comorbidities, n (%)	0	3 (9.37)	16 (24.24)	14 (20.89)	0.273[†]
	1	9 (28.12)	23 (34.84)	30 (44.77)	
	2	14 (43.75)	16 (24.24)	16 (23.88)	
	3	3 (9.37)	7 (10.6)	5 (7.46)	
	4	3 (9.37)	4 (6.06)	2 (2.98)	
CCI, median (range)		2 (1–5)	1 (1–4)	1 (1–6)	0.003[+ab]
MCCI, mean ±SD		5.22 ±1.69	4.06 ±1.71	3.6 ±1.75	0.000*[ab]
PN, n (%)	present	24 (75)	51 (77.27)	39 (58.20)	0.043[†a]
	absent	8 (25)	15 (22.72)	28 (41.79)	
PAD, n (%)	present	27 (84.3)	33 (50)	26 (38.8)	0.000[†ab]
	absent	5 (15.6)	33 (50)	41 (61.1)	
Smoking history, n (%)	present	10 (31.25)	14 (21.21)	11 (16.41)	0.240[†]
	absent	22 (68.75)	52 (78.78)	56 (83.58)	
Hypertension, n (%)	present	18 (56.25)	34 (51.51)	32 (47.76)	0.726[†]
	absent	14 (43.75)	32 (48.48)	35 (52.23)	
IHD, n (%)	present	10 (31.25)	25 (37.87)	22 (32.83)	0.754[†]
	absent	22 (68.75)	41 (62.12)	45 (67.16)	
Nephropathy, n (%)	present	10 (31.25)	11 (16.66)	11 (16.41)	0.168[†]
	absent	22 (68.75)	55 (83.33)	56 (83.58)	
Hemodialysis, n (%)	present	8 (25)	5 (7.57)	7 (10.44)	0.040[†a]
	absent	24 (75)	61 (92.42)	60 (89.55)	
Cost [USD], median (range)		1023 (228–9362)	535 (111–12,852)	762 (56–13,358)	0.032[+a]

SD – standard deviation; DFU – diabetic foot ulcer; DM – diabetes mellitus; CCI – Charlson Comorbidity Index; MCCI – modified CCI; PN – peripheral neuropathy; PAD – peripheral arterial disease; IHD – ischemic heart disease; * analysis of variance (ANOVA) test; † χ^2 test; [+] Kruskal–Wallis test; [a] difference between group 1 and group 2 was statistically significant; [b] difference between group 1 and group 3 was statistically significant.

length of hospital stay, DFU duration, Wagner stage, CCI, and MCCI were determined as the variables affecting the cost, and the results of multiple linear regression analysis showed that only the length of stay had a significant relationship with cost (p = 0.000) (Table 5).

Discussion

This study examined the epidemiological factors that may be effective in determining the prognosis of DFU patients grouped into major amputation, minor amputation

Table 2. Comparison of laboratory results of groups

Variables	Group 1 (n = 32)	Group 2 (n = 66)	Group 3 (n = 67)	p-value
HbA1c (%), mean ±SD	9.26 ±2.62	9.48 ±2.31	9.25 ±2.28	0.827*
WBC [10^3/µL], median (range)	15.12 (7.48–33.76)	11.54 (5.54–32.29)	8.89 (4.09–32.73)	0.000^{+ab}
ESR [mm/h], mean ±SD	77.03 ±33.04	59.36 ±33.32	46.72 ±30.88	0.000*^{b}
CRP [mg/L], median (range)	177.6 (22.2–393.5)	69.5 (1.4–369)	43.5 (0.3–361)	0.000^{+ab}
PCT [µg/L], median (range)	0.01 (0.001–100)	0.80 (0.001–13)	0.06 (0.02–15)	0.000^{+b}
Creatinine [mg/dL], median (range)	1.23 (0.51–10.3)	1.08 (0.51–11.55)	1.02 (0.42–6.6)	0.380^{+}
BUN [mg/dL], median (range)	25.05 (9.7–100.5)	22.65 (7–108)	22 (5.7–61.4)	0.042^{+b}
ALB [g/L], mean ±SD	28.42 ±6.91	34.28 ±5.72	35.39 ±6.57	0.000*^{ab}

HbA1c – glycated hemoglobin A1c; SD – standard deviation; WBC – white blood cell; ESR – erythrocyte sedimentation rate; CRP – C-reactive protein; PCT – procalcitonin; BUN – blood urea nitrogen; ALB – albumin; * analysis of variance (ANOVA) test; $^{+}$ Kruskal–Wallis test; ^{a} difference between group 1 and group 2 was statistically significant; ^{b} difference between group 1 and group 3 was statistically significant.

Table 3. Isolated microorganisms and their characteristics

Microorganism	Group 1 (n = 19)	Group 2 (n = 52)	Group 3 (n = 45)	Total (n = 116)
Gram-positive bacteria, n	5	18	21	44
Staphylococcus aureus	2	5	100	17
Enterococcus faecalis	0	4	5	9
Coagulase negative staphylococci	2	4	2	8
Streptococcus agalactiae	1	2	2	5
Dermacoccus nishinomiyaensis	0	1	0	1
Diphtheroid bacillus	0	1	0	1
Kocuria rhizophila	0	0	1	1
Streptococcus thoraltensis	0	0	1	1
Enterococcus avium	0	1	0	1
Gram-negative bacteria, n	14	32	23	69
Escherichia coli	4	4	5	13
Pseudomonas aeruginosa	2	5	4	11
Acinetobacter baumannii	1	6	2	9
Proteus mirabilis	1	3	3	7
Enterobacter cloacae	0	5	1	6
Klebsiella pneumoniae	1	2	2	5
Morganella morganii	1	2	2	5
Klebsiella oxytoca	0	2	2	4
Citrobacter freundii	2	0	0	2
Klebsiella aerogenes	0	2	0	2
Stenotrophomonas maltophilia	1	0	0	1
Citrobacter braakii	1	0	0	1
Acinetobacter lwoffii	0	0	1	1
Serratia rubidaea	0	0	1	1
Proteus hauseri	0	1	0	1
Other microorganisms, n	0	2	1	3
Skin flora	0	0	1	1
Fungi	0	2	0	2

Table 4. Evaluation of risk factors for amputation according to multinomial logistic regression analysis

Variables		Regression coefficient	p-value	OR	95% CI
Major amputation group	male sex	7.110	0.345	1223.778	0.000–3.11
	age	2.708	0.016	15.005	1.645–136.847
	Wagner classification	15.359	0.004	46.822	0.147–148.6
	insulin use	–1.221	0.347	0.295	0.023–3.763
	duration of DFU	0.773	0.048	2.167	0.996–4.715
	number of comorbid diseases	0.052	0.923	1.054	0.365–3.045
	CCI	2.015	0.046	7.503	0.930–60.526
	MCCI	–1.867	0.144	0.155	0.013–1.898
	ALB	–0.122	0.213	0.885	0.730–1.073
	HbA1c	–0.145	0.504	0.865	0.566–1.322
	CRP	0.009	0.105	1.009	0.998–1.019
	WBC	0.001	0.010	1.001	1.000–1.001
	ESR	0.012	0.402	1.012	0.984–1.041
Minor amputation group	male sex	–18.648	0.000	7.96	–4.02–0.0
	age	1.070	0.042	2.916	0.981–8.674
	Wagner grade	2.087	0.031	8.064	1.207–53.859
	insulin use	–0.494	0.314	0.610	0.234–1.595
	duration of DFU	–0.011	0.956	0.989	0.661–1.480
	number of comorbid diseases	0.057	0.835	1.059	0.619–1.810
	CCI	–0.598	0.392	0.550	0.140–2.164
	MCCI	–0.332	0.603	0.717	0.205–2.513
	ALB	0.037	0.411	1.038	0.950–1.133
	HbA1c	0.175	0.131	1.191	0.949–1.493
	CRP	–0.004	0.321	0.996	0.990–1.003
	WBC	0.000	0.265	1.000	1.000–1.000
	ESR	0.019	0.022	1.019	1.003–1.036

OR – odds ratio; 95% CI – 95% confidence interval; DFU – diabetic foot ulcer; CCI – Charlson Comorbidity Index; MCCI – modified CCI; ALB – albumin; HbA1c – glycated hemoglobin A1c; CRP – C-reactive protein; WBC – white blood cell; ESR – erythrocyte sedimentation rate.

and non-amputation groups, and compared the costs associated with each group. The results showed that high acute phase protein values and low ALB levels in group 1 patients, as well as the presence of high Wagner grades, PN and PAD were significant. Furthermore, the Doppler USG examinations demonstrated that the rate of distal vessel involvement was high in group 1 patients.

Although *Staphylococcus aureus* was the most common infectious agent, Gram-negative pathogens were dominant in all 3 groups. There was also a significant difference in cost between the groups, with hospital stay length being the main factor affecting the cost. Furthermore, age >65 years, low ALB values, high Wagner grade, high CCI, long DFU duration, and high WBC count were identified as risk factors for major amputation.

Diabetic foot ulcer is associated with high morbidity and mortality, and is one of the potentially preventable complications of diabetes.[7,13] A wide variety of diabetic foot amputation risk factors have been reported in previous studies.[4,13] Such diversity may be due to differences in study subjects and designs, treatment protocols and cultural characteristics.[4,11–13]

Various studies have produced different results on whether there is a significant relationship between age and amputation.[2,4,6,7,9,12,13] As people age, the wound healing process progressively deteriorates due to many factors, such as impaired defense mechanisms and immunity and the development of PAD.[6,13] In this study, advanced age was an important determinant, with the mean age of the patients who underwent major amputation being significantly higher than in the other groups. Moreover, advanced age increased the risk of major amputation 15-fold and the risk of minor amputation approximately 3-fold in DFU patients.

Gender, smoking, age, and DM duration are prognostic factors for amputation. They have been evaluated in the previous studies, though the results are controversial.[1,6,7,9,12,22–24] Although there was no statistically significant relationship in terms of gender between the groups in the current study, the risk analysis indicated that being male increased the risk of minor amputation approximately

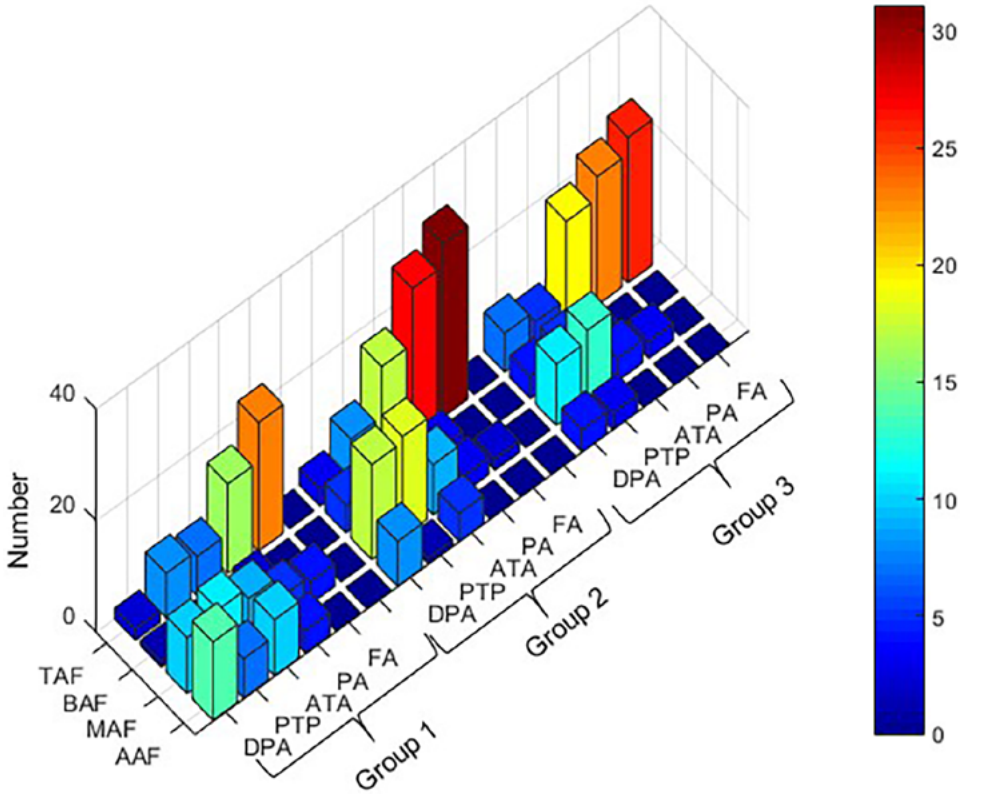

Fig. 1. Distribution of flow phases detected using Doppler ultrasonography in patients with peripheral artery involvement

AAF – absence of arterial flow;
ATA – anterior tibial artery;
BAF – biphasic arterial flow;
DPA – dorsalis pedis artery;
FA – femoral artery;
MAF – monophasic arterial flow;
PA – popliteal artery;
TAF – triphasic arterial flow;
PTP – posterior tibial artery.

8-fold. On the other hand, although the major amputation patients had a longer mean DM duration, there was no significant relationship between the groups. Furthermore, smoking was not identified as a risk factor for LEA in this study.

In the current study, longer DFU duration was significantly associated with major amputation, which increases the risk of wound infections that can result in tissue necrosis. Such infections cause irreversible damage, with deep tissue involvement depending on the processes observed, and increase the risk of complications.[6–9,24,25] In this regard, major amputation risk nearly doubled as the DFU duration increased.

Levels of HbA1c are directly related to the mean glucose concentration over the Hb lifetime,[6,8] and the primary risk factor for developing diabetic complications is poor glycemic control.[2,6,8] According to several studies, the HbA1c level is a predictor of amputation.[2,6,24] However, the current study found no significant difference in HbA1c levels between the groups.

Individuals with DM are more likely to have PAD,[13,25,26] which is a substantial risk factor for LEA.[8,9,12,13,25] Ulcers become complicated due to ischemia, which occurs when PAD causes insufficient blood flow for ulcer healing.[13,25,27] Furthermore, wound granulation and healing require adequate nutritional support to the tissues.[25–27] In the presence of PAD, the concentration of tissue antibiotics decreases, and the risk of multidrug-resistant microbes multiplying in DFUs becomes greater, thereby increasing the possibility of amputation.[25–27] In the current study, there was a significant difference in PAD incidence between the groups, with 84% in the major amputation group, 50% in the minor amputation group and 38% in the non-amputation group. In group 1, group 2 and group 3, the incidence of monophasic flow or absence of flow in the dorsalis pedis artery was 95%, 80% and 60%, respectively. Meanwhile, distal artery involvement was more common in the major amputation group. These findings demonstrate that as PAD incidence and severity increase, so do the rate and level of amputation.

Peripheral arterial disease, DFU depth and presence of infection are the most commonly used parameters for DFU classification.[2,8,13,28,29] It has been shown that the Wagner classification,, the most common classification system used to describe DFU characteristics, is effective for prognosis.[2,8,13,28,29] However, its sensitivity in predicting LEA is 93.6%, and its specificity is 50.8%.[2,8,28] In this study, major amputation patients were classified as Wagner grade 4 or 5, and lower grades were detected in patients with minor amputations and those who did not undergo amputation, with a significant difference between them. Being classified as Wagner grade 4 or 5 increased the risk of major amputation approx. 47 times and the risk of minor amputation 8 times.

Since the CCI includes diabetes severity, PAD status and nearly all independent amputation risk factors, a high score of this index is an amputation indicator and can be used as a clinical tool.[19–21,30,31] There was a significant difference in CCI and MCCI between group 1 and the other 2 groups. In the risk analysis, a CCI ≥ 4 increased the risk

Table 5. Factors determining the cost (with multiple linear regression analysis)

Variables	B	SE	β (95% CI)	t	p-value
		Group 1			
Age	–250.554	4638.022	–0.009	–0.054	0.957
Sex	235.045	4082.116	0.008	0.058	0.955
Length of hospitalization	7597.503	1785.390	0.673	4.255	0.000
Duration of DFU	–552.367	2021.895	–0.048	–0.273	0.787
Wagner grade	900.469	5782.375	0.025	0.156	0.878
CCI	–4094.475	4248.726	–0.176	–0.964	0.345
MCCI	5196.98	5708.88	0.197	0.910	0.372
		Group 2			
Age	–666.167	2873.942	0.028	0.232	0.818
Sex	–4748.349	2604.285	–0.194	–1.823	0.073
Length of hospitalization	5938.442	1141.558	0.543	5.202	0.000
Duration of DFU	–199.733	1309.653	–0.017	–0.153	0.879
Wagner grade	391.490	2621.614	0.016	0.149	0.882
CCI	7127.234	4394.209	0.199	1.622	0.110
MCCI	–4748.349	3272.719	0.023	0.165	0.870
		Group 3			
Age	–4667.808	4569.127	–0.150	–1.022	0.311
Sex	701.266	3855.325	0.021	0.182	0.856
Length of hospitalization	7105.261	1719.424	0.509	4.132	0.000
Duration of DFU	734.218	1519.840	0.057	0.483	0.631
Wagner grade	–1196.057	10618.4	–0.013	–0.113	0.911
CCI	–1489.57	4641.056	–0.042	–0.321	0.749
MCCI	5667.4	5107.638	0.174	1.110	0.272
		Total			
Age	–1566.719	2143.609	–0.58	–0.731	0.466
Sex	–1394.648	1907.803	–0.048	–0.731	0.466
Length of hospitalization	6738.996	836.049	0.543	8.061	0.000
Duration of DFU	241.870	825.283	0.020	0.293	0.770
Wagner grade	–346.083	2006.335	–0.012	–0.172	0.863
CCI	412.549	2347.424	0.013	0.176	0.861
MCCI	4252.942	2426.024	0.154	1.753	0.082

β – standardized coefficients; B – unstandardized coefficients; 95% CI – 95% confidence interval; DFU – diabetic foot ulcer; CCI – Charlson Comorbidity Index; MCCI – modified CCI; SE – standard error.

of major amputation 7.5 times. There was no significant difference in the number of comorbidities between the groups. However, the specific disease, disease stage and the extent of its effect on the tissues in DFUs play a greater role in the prognosis than the number of comorbidities. Therefore, the quality of the accompanying diseases rather than their quantity is a crucial determinant of the level of amputation.

Diabetes mellitus and CRF have important common risk factors that predispose to DFU formation, such as PN, PAD and susceptibility to infection.[2,4,9,32] Moreover, CRF is considered an indicator of future PAD,[2,4,9,32] and a significant association has been established between the deterioration of kidney function and DFU recurrence and amputations.[4,9,22,23] However, a meta-analysis found that nephropathy was not the cause of amputation in patients with a diabetic foot infection, despite its role in the development of DFUs.[2] Furthermore, it has been reported that nephropathy may not be a direct indicator of amputation, as the predictive value of different nephropathy stages may vary.[2,9]

In this study, 19.3% of the patients were diagnosed with CRF, and 62.5% were undergoing hemodialysis. There was no significant relationship between the groups in terms of CRF. However, there was a significant difference between group 1 and group 2 in the proportion of patients undergoing hemodialysis. Therefore, it can be concluded that there may be a possible increase in the number of major amputations in DFU patients as the CRF stage increases.

Peripheral neuropathy is one of the major risk factors for all foot complications.[6,12] In addition to foot deformity caused by PN, neuropathic changes, such as decreased protective sensation and skin cracks due to decreased sweating, lead to the formation of diabetic foot infections.[5,6] Furthermore, the healing of DFUs can occur without complications in patients without PN.[6] Peripheral neuropathy was present in 69% of the patients in this study, and there was a significant difference in PN incidence between group 1 and 2.

Diabetic foot ulcer treatment requires specialist care, orthopedic tools, antimicrobial drugs, various dressing materials, and inpatient care,[1,4,9] which leads to a significant economic burden.[1,4,9,13] The cost of DFU treatment to the healthcare system varies by country,[1] though DFU treatment accounts for approx. 25% of the total hospital costs for a diabetic patient.[1,2,4,13] In the current study, group 1 had the highest mean treatment cost, followed by group 3 and group 2, respectively. The reason for the high cost in group 3 patients is likely due to the extended hospital stay and the dressing equipment used. Meanwhile, the factor that increased the treatment cost of patients who underwent major amputation was hospitalization in the intensive care unit (ICU) after surgery. According to the regression analysis, the length of hospital stay was the only factor affecting the cost, though the costs do not fully represent the total economic burden. Indeed, when associated costs, such as loss of productivity, clinical follow-up, rehabilitation, and home care, are taken into account, higher costs may be encountered.

C-reactive protein and ESR levels and WBC count are the most frequently used parameters for detecting infection in clinical practice,[2,8,9,33] and are useful for showing changes in disease activity.[2,5,9,33] In this study, mean WBC count, CRP level, ESR rate, and PCT values were significantly higher in group 1 than in groups 2 and 3.

Proteins are vital for matrix synthesis and healing at the wound site.[8,34] It has been reported that patients with ALB levels greater than 28–35 g/L recovered without complications.[8,34] In this study, the mean preoperative ALB values were 28.4 g/L (group 1), 34.2 g/L (group 2) and 35.3 g/L (group 3). The comparison of the ALB values between groups showed a significant difference, which is consistent with the supporting literature.[8,34]

Approximately 56% of DFUs are infected, and 20% of them require amputation.[2,5,9,12] Although Gram-positive pathogens, especially *Staphylococcus* spp., are seen more frequently in diabetic foot infections, others have reported detecting Gram-negative pathogens more often.[2,5,9,12] Gram-negative bacteria isolation poses a higher risk of amputation than Gram-positive bacteria isolation,[2,5,9] although *S. aureus* is reported to be a predictor of limb loss.[5,9,12] In this study, Gram-negative microorganisms were most common in all 3 groups. Nonetheless, considering the results of all cultures, *Staphylococcus* spp. were the most common causative microorganisms. Meanwhile, Gram-negative pathogens were predominantly detected in group 1 and 2 patients, and Gram-positive and Gram-negative pathogens were found at an almost equal frequency in group 3.

Early diagnosis and treatment of DFUs are vital due to the increasing prevalence of diabetic patients and the subsequent increased burden on healthcare system and costs. Moreover, improved management of diabetic patients in the initial stages is crucial, as the severity of the condition increases when complications arise. Therefore, identifying risk factors in DFU patients will help to develop effective strategies for diagnosis, management and treatment protocols. We believe that increasing knowledge in the DFU field through the current and similar studies will help define risk assessment models that can be used in clinical practice.

Limitations

This study had several limitations. Although the data were collected prospectively, the study was retrospective in design, meaning that the findings need to be confirmed in prospective studies. Also, the sample size is relatively small, though it is more than sufficient compared to similar studies. Furthermore, stepwise selection methods are widely applied to identify covariates for inclusion in regression models, which leads to biased estimation of the regression coefficients and can cause a significant bias in the estimated regression coefficients. Finally, the study was undertaken in a developing country and may not reflect DFU patients in developed countries.

Conclusions

This study demonstrated high Wagner grades, PN and PAD incidence in major amputation patients. Furthermore, age >65 years, long DFU duration, low ALB values, high Wagner score, increased CCI, and elevated WBC count were risk factors for major amputation. Although *S. aureus* was the most common infectious agent, Gram-negative pathogens dominated. Moreover, major amputation patients had a high rate of distal vessel involvement, higher acute phase protein levels and lower ALB levels. There was also a significant difference in cost between the groups, and the most important factor was the length of hospital stay.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.7826090. The package contains the following files:

Supplementary linear regression tests file.
Supplementary normality tests file.
Supplementary multinomial logistic regression test results.
Supplementary normality test table.

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